RESISTANCE OF THE PURPLE SEA URCHIN TO OSMOTIC STRESS[1]

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The western purple sea urchin, *Strongylocentrotus purpuratus,* is briefly exposed to drastic environmental changes during the very low tides which occur in summer and winter along the central California coast. On such occasions these animals may be subjected to dehydration, due to high temperature, direct exposure to the sun, and particularly the wind. During heavy rains at low tides they may be exposed to considerably diluted sea water. The sea urchin is thus exposed to a wide range of salinities in the intertidal area. The osmotic tolerance of these animals was therefore determined by observations and experiments reported in this paper. During winter months when tide-pool water is diluted by rains, spawning may be observed; therefore, the influence of salinity changes on fertilization and development was also examined. The results reported in this paper suggest that sea urchins and their developing embryos can tolerate considerably greater variations in osmotic conditions than they are likely to meet in their environment, indicating an ample "safety factor" in their constitution.

Materials and Methods

The sea urchins were collected at low tides, primarily near Yankee Point, five miles on the Pacific Coast south of Carmel, California. At this point large populations of the purple sea urchin are exposed at low tides. Other collections were made at Moss Beach, California, near Stanford University. At the Hopkins Marine Station the urchins were put into aquaria with running sea water at a temperature varying between 12 and 16.7° C. At the University they were kept in a constant temperature room at 13° C. in well-aerated sea water, changed daily, until the specimens were used.

The resistance of the adult sea urchins to changes in salinity had to be assessed by their reactions and appearance. Healthy sea urchins, if not too crowded, tend to crawl up on the sides of aquaria rather than stay at the bottom, while unhealthy animals usually remain on the bottom. Healthy urchins eat algae avidly, sickly ones do not. Healthy urchins, when overturned, right themselves in a coordinated manner within about a minute, whereas unhealthy ones may fail to do so, or take more time. Stimulation of normal animals with a probe or a bright spot of light (*e.g.,* from an American Optical Company Universal Illuminator) brings about a positive reaction (local erection, in the direction of stimulation, of spines and pedicellariae) and stronger stimulation elicits an escape reaction. These responses are abnormal in unhealthy animals. Finally, animals which are sick or dying

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lose pigment and spines. Detection of slight changes in appearance or behavior of these animals is somewhat subjective, but marked changes are very definite and easy to note. Failure to show any response to prodding indicates that an animal is, to all intents and purposes, dead.

It proved impossible to develop a consistently reliable source of running sea water at various salinities because of difficulties with regulating valves and air embolism in the tubes. The various solutions to be tested were made up in 7-liter battery jars, using appropriate mixtures of sea water, tap water and saturated brine (from the salt pools at Moss Landing, California), and aerated vigorously. Eight to 10 animals, 3.0–3.5 cm. in test diameter and totaling about 200 grams wet weight, were kept in each jar. The concentrations of sea water tested were 61.5%, 70.4%, 80.7%, 90.3%, 110%, 121.4%, 130.8%, 150% and 170% as determined by titration with silver nitrate, using potassium chromate as indicator.

In the developmental studies at different salinities the following procedures were observed: Ovaries were removed from gravid female sea urchins, avoiding contamination with other tissues, and suspended in filtered sea water in Syracuse watch glasses. The concentrated egg suspension was picked up in a mouth pipette under a dissecting microscope and added, with a minimum of sea water, to about 1 ml. of the appropriate hypertonic or hypotonic sea water (made up with distilled water). Sperms were added in small quantities from the tip of a needle, and the progress of fertilization, first and second cleavages, early and late blastulation, gastrulation, and in some instances, pluteus formation, was noted.

Changes in the osmotic pressure of the perivisceral fluid of the sea urchins exposed to sea water of various tonicities were determined by the method of Gross (1954). The effects of tonicity changes of sea water on the respiration of sea urchins were studied with the standard Warburg-Barcroft manometric method, using large respirometric flasks which readily accommodate small sea urchins.

In all experiments small sea urchins were employed in order to have a more homogeneous population sample. For respirometry they had to be 2–3 cm. in diameter to fit into the flask (Farmanfarmaian, 1959). In the other experiments specimens 3.0–5.5 cm. in diameter were used. Experiments were carried out soon after the animals were collected and when they were all well fed and healthy. Feeding was avoided during experiments (except when testing the feeding reaction) to prevent fouling the aquaria. The latter had to be cleaned frequently at first because of extensive defecation. When an animal died it was promptly removed, since all the other animals in a container will soon die unless this is done.

Experimental Results

1. *Osmotic tolerance of sea urchins*

It is apparent from the results in Table I that the extreme salinities are almost immediately injurious; no response to stimulation was obtained after a three-hour exposure of sea urchins to 30%, 50%, 150% and 170% sea water. Those in 60% and 130% sea water, which also showed no response after three hours, recovered when replaced in sea water. Most of the studies were concerned with the other concentrations, close to sea water, because they lie within the range of greater ecological interest.

TABLE I

Osmotic tolerance of adult Strongylocentrotus purpuratus*

Per cent sea water	Effect of short exposure (3 hrs.)	Effect of prolonged exposure (days)
30	No activity or response; no recovery (die)	—
50	No activity or response; recover later**	Die by second day.
60	Little activity or response; recover later**	Lose much pigment; unresponsive; most of them die by the second day.
70	Normal at beginning	Stay near bottom of container; some die on 5th day, all by 25th day.
80	Normal***	Lose some pigment for first three days; survive†—35 days.
90	Normal***	Lose a little pigment for first three days; survive†—35 days.
100	Normal***	Normal; survive†—35 days.
110	Normal***	Lose some pigment continuously; some reduction in activity; survive†—35 days.
120	Normal at beginning, reduced response; recover later**	Lose pigment, stay near bottom of container; some dead by 7th day; half are dead by the 35th day.
130	Little activity or response; recover later**	Lose pigment; die on second day.
150	No activity or response; no recovery	—
170	No activity or response; no recovery	—

* Temperature varied between 12.0 and 16.7° C. during the course of these experiments.
** When replaced into sea water (100%).
*** For the entire period of observation—35 days.
† Climb up sides of container (at least at first); right themselves rapidly; feed upon *Iridaea* (red alga); respond to bright light and touch.

Although the sea urchins tolerated 70% and 120% sea water for three hours, they were not normal after a more prolonged exposure and they stayed near the bottom of the tank instead of climbing along the sides as did the controls. They then lost pigment and many died in 70% and 120% sea water between the twenty-fifth and thirty-fifth day of exposure. These concentrations of sea water, therefore, constitute the limits of tolerance. Tonicities of 80%, 90% and 110% sea water were tolerated, the animals remaining essentially normal for at least 35 days (in some cases to 50 days) of exposure and observation. Needless to say, controls in sea water remained normal for much longer periods.

The response to changes in the concentration of sea water is tolerance and not regulation. This is indicated by the change in weight of sea urchins following immersion in sea water of diverse tonicities, as seen in Figure 1A for one series of experiments. The animals used were 3.0 to 5.0 cm. in test diameter and weighed 25 to 35 grams. They were immersed in 500 ml. of the appropriate solution in a 600-ml. beaker and drained on a towel for 5 minutes before weighing. The change in weight was calculated as per cent of original weight. Figure 1B shows the change in the osmotic pressure of the perivisceral fluid as a result of immersion

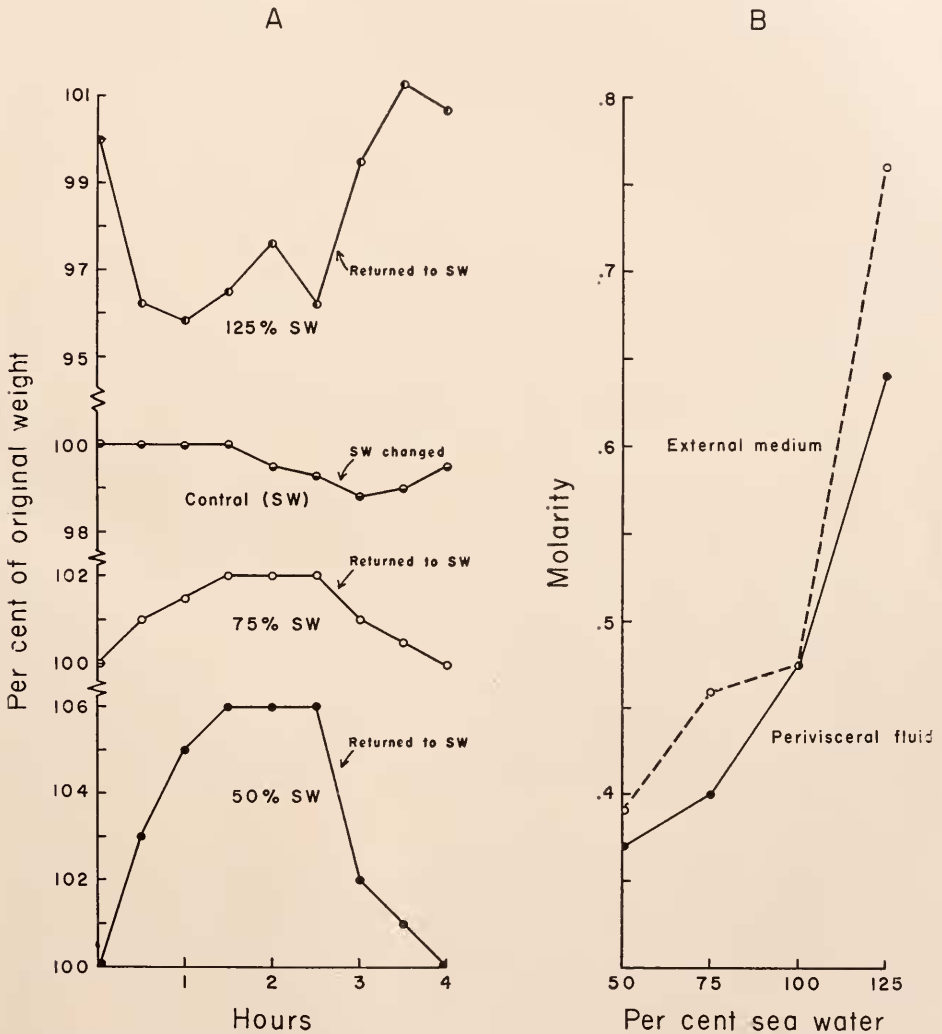

FIGURE 1. A. Changes in weight of sea urchins placed in various concentrations of sea water and again after replacement in sea water. B. Changes in concentration of perivisceral fluid at equilibrium (after a 1.5-hour exposure of the sea urchin to the solution); concentration is given in terms of NaCl equivalents. Note that the perivisceral fluid approximates the external bathing medium in concentration.

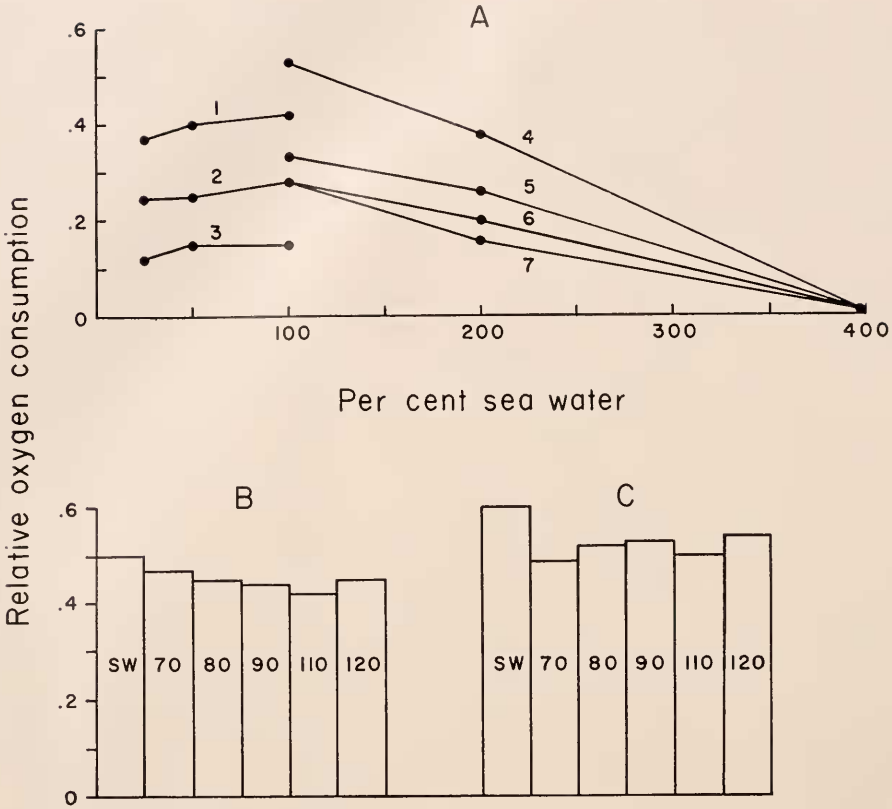

FIGURE 2. A. Relative change in oxygen uptake of sea urchins placed in various concentrations of sea water. The changes in respiration at various concentrations of sea water were studied in the order indicated, starting with each sea urchin in normal sea water. The initial oxygen consumption recorded is characteristic for each sea urchin, depending upon its size and other characteristics. B and C. Histograms of changes in oxygen consumption of two sea urchins with changes in concentration of sea water within the range of tolerance. Similar results (omitted here) were obtained with three other specimens. In all cases, slight changes in oxygen uptake were observed with variations in concentration of the bathing sea water. The initial oxygen uptake of each specimen depends upon its size and other characteristics. Comparisons should be made for the same sea urchin at different concentrations of bathing sea water.

of the urchins in sea water of diverse tonicities. In each test the capillaries used for the melting point tests were partially filled with either the bathing fluid or the perivisceral fluid. The latter was obtained by inserting the capillary directly through the peristomial membrane into the main coelom. Care was taken not to contaminate such samples with the bathing fluid. The results in Figure 1B show that the osmotic pressures of the perivisceral fluid and external media are about the same once equilibrium is established, *i.e.*, no change in weight is observed. The differences observed at 75% and 125% sea water are probably due to incomplete equilibrium.

Animals which regulate the salt concentration of their body fluids expend

metabolic energy and often increase their metabolic rate under conditions of osmotic stress (Prosser and Brown, 1961). The respiration of sea urchins exposed to various salinities was measured, to determine whether changes in salinity altered the respiratory rate. The respiratory rate for each individual was first measured in normal sea water. This water was then aspirated and replaced by the test solution, and determinations were started only after 40 minutes of equilibration. In longer experiments one or more controls were used to safeguard against possible changes due to internal clocks or other uncontrollable factors. The values obtained for experimental animals were then corrected by the changes in these controls (for methods, see Farmanfarmaian, 1959).

The data shown in Figure 2A for three series of experiments indicate that there is no marked change in respiration even when the urchins are immersed in 50% sea water, and only a small decrease when they are in 25% sea water, and most marked in 400% sea water.

Within the tolerance range (70% to 120% sea water) the variation in respiration is indeed very slight as shown by 5 series of experiments. Only two of these experiments are shown in Figures 2B and C.

To determine whether the osmotic stress of diluted sea water could be relieved by addition to the medium of an inert, non-penetrating organic compound, sea urchins were subjected to 50%, 25% and 5% sea water made equivalent to sea water in tonicity by the addition of one molal sucrose (A. Klein and R. Rasmussen, unpublished data), much in the manner of Loeb's study on a crustacean in 1903. After two hours the sea urchins appeared dead by the response criteria (see Methods). Tests of the body fluid chlorinity by silver nitrate titration (using silver chromate as indicator) showed some loss of salts from the body fluid. Tests for reducing sugar in the body fluid (after hydrolysis with HCl) were negative, indicating no marked entry of sucrose (the method would not have detected slight entry). Sea urchins, after two hours in each of these media, replaced in aerated sea water responded and appeared normal after 24 hours. At this time body fluid osmotic pressure (method of Gross, 1954) was normal and the weight, which had initially dropped, was essentially back to normal (slightly greater). When sea urchins were placed directly in one molal sucrose, irreversible damage occurred within two hours. Tests now disclosed more rapid loss of salts and marked entry of sucrose, suggesting that the cells lining the sea urchin membranes had died, and that equilibrium was being established between the body fluid and the external medium.

The experiments do not tell what is happening to the cells of sea urchins placed in diluted media. Perhaps salt losses in them are much quicker and more marked than can be detected in the massive body fluid which was tested, and loss of excitability may well follow such cellular depletion. In any case, sucrose protects against dilution of sea water to only a limited degree, indicating that the problems which the sea urchin meets in media of altered osmotic pressure are ionic as well as osmotic.

2. *Osmotic tolerance of sea urchin eggs and developmental stages*

The results of tonicity changes on the development of sea urchin eggs are shown in Table II. Eggs immersed in 200% sea water perceptibly crinkled (due to loss

TABLE II

Osmotic tolerance of developmental stages of Strongylocentrotus purpuratus

Per cent sea water	Fertilization membrane	Cleavage* to 2 cells	Cleavage* to 4 cells	Blastulae*	Gastrulae*
50 + 0.5 *M* sucrose	tight	normal	normal	almost like control	almost like control
60	about half; eggs swell	occasional, abnormal, delayed	abnormal delayed	abnormal, multicellular masses	—
70	all eggs** swell	much like 80% but more exaggerated	delayed	much delayed and abnormal	—
80	all	much like controls, blastomeres rounded	delayed	delayed, but good	abnormal fragmenting
90	all	much like controls	seemingly normal	like control	like control
100	all	normal	normal	normal	normal
110	all	most cleave	seemingly normal	like control	like control
120	all	most cleave, delayed	delayed	delayed, small	many quite abnormal
150	about a fifth; sperms active	a few cleave	delayed	some blastulae***	—
100 + 0.5 *M* sucrose	sperms immobilized	some cleave	delayed	abnormal	—
200	none; sperms immobilized; eggs shrink	—	—	—	—

* The time table for cleavage of eggs of *S. purpuratus* at 13° C. has been given elsewhere (Giese, 1938). The first cleavage requires almost two hours at 13° C., the subsequent cleavages occur at about hourly intervals. Free-swimming blastulae are found 20 hours after insemination, gastrulation occurs in about 32 hours after insemination, and the gut begins to differentiate in 46 hours after insemination. Plutei form in about 96 hours, although the arms do not appear until about 120 hours after insemination.

** "Like controls"—usually a few eggs in both control and experimental series do not develop fertilization membrane and fail to develop.

*** In one series some abnormal blastulae developed; in one, only abnormal masses of nonmotile cells.

of water), the sperms were immediately immobilized, and failed to penetrate the eggs. Later examination showed no signs of fertilization or cleavage. In 150% sea water the sperms were highly motile and about 20% of the eggs mixed with sperms developed fertilization membranes, but such cleavages as occurred were delayed and quite abnormal. In 120% sea water the eggs fertilized to the extent that controls did, but cleavages were delayed and resulted mostly in abnormal balls of cells. However, 24 hours later some blastulae became motile. Eggs in 60% sea water were also so damaged that only about half of them showed fertilization membranes, and only about 20% cleaved; none formed blastulae. In 70% sea water early cleavage was observed but later cleavages were much delayed and abnormal. The blastomeres of cleaving eggs were more rounded and larger than the controls in sea water. Eggs at the other concentrations (80%, 90%, 100% and 110% sea water) developed normally to the early blastula stage, and 24 hours later all except those in 80% sea water formed swimming blastulae. The majority of the embryos in 80% sea water were definitely unhealthy although a few normal free-swimming blastulae appeared at the same time as in the controls. Those in 90% sea water appeared even healthier than controls in sea water.

Twenty-four hours later, gastrulae appeared in the 110%, 100% and 90% sea

water; in one of 8 trials with 80% sea water, gastrulae appeared but they were not normal.

It is interesting, however, that blastulae and gastrulae transferred from the control in sea water to 70%, 80%, 120% and 150% sea water were alive and healthy 8 hours later. Those in 150% sea water were smaller than controls, and each had a shorter gut and a dense mass of cells around it. All survived for a week, at which time the experiment was terminated. At this time controls were in the late prism stage with skeletal rods and well-developed gut just preceding formation of the pluteus. Only the embryos in 110% sea water showed a degree of development comparable to the controls. Those in 90% sea water were almost as well developed but neither the skeletal rods nor the gut were comparable to those in the controls. Those in concentrations far to either side of sea water remained as enlarged and undifferentiated gastrulae, although in some of them a mouth opening appeared. Thus, it is apparent that not only the early development, but also the later development of the embryos is adversely affected by tonicities of sea water much removed from the normal medium.

Changes in the development of sea urchin eggs observed in the hypotonic and hypertonic sea water discussed above are apparently due to changes in osmotic pressure rather than the quantity of the salts. This proposition was borne out by a series of experiments in which 50% sea water was made isosmotic with sea water by addition of approximately 0.5 molal sucrose (see Loeb, 1908; Harvey, 1956). The various stages of development from fertilization to the advanced gastrula are essentially normal in such a solution (see Table II). The rate of development and the percentage of zygotes attaining the advanced gastrula stage also are comparable to controls. It must be stated, however, that embryos developing in such a solution appear to be a little more compact than the controls. Also, when control blastulae are transferred to this sucrose-sea water medium they lose water at first but soon attain equilibrium and appear normal thereafter.

There is a limit, however, to which sucrose may be substituted for the salts of sea water without altering development. Thus, when 5% sea water is made isosmotic by the addition of sucrose, sperms are quickly immobilized and the fertilization rate drops drastically. It is known that changes in the solute environment alter the permeability of cells (Lucké, 1940). Development of the few eggs which are fertilized is completely abnormal. Also normal blastulae transferred to such a solution became abnormal within 6 hours. The minimal salt requirements for normal development of *S. purpuratus* are being studied (Deboyd Smith, unpublished). Extensive studies of this type were made by Herbst (1903) on eggs of European sea urchins.

The effects of a 0.5 molal sucrose solution made up in sea water were similar to those observed in 150% sea water. The sperms were quickly immobilized, and only a small percentage of the eggs were fertilized. The zygotes formed very compact abnormal blastulae, and normal blastulae transferred to the hypertonic solution swam actively for 12 hours but became quite abnormal.

Discussion

Echinoderms are typically marine animals but a few are found in brackish water, for example, *Asterias rubens,* which occurs in the Baltic Sea (Schlieper,

1956). This sea-star may live in a salinity as low as 8 parts per thousand (8‰) in the middle Baltic near Rügen. It can be moved from 8‰ to higher salinities without damage, and from higher salinities to lower ones—but only gradually—permitting progressive adaptation over a period of several weeks. While the sea-stars tolerate such lowered salinities, it is interesting to note that they grow to a smaller size and show changes in many characteristics. For example, in water at 15‰—as compared to 30‰—righting reactions are slower, the gonads develop more slowly (although to the same extent), the tissue metabolism is decreased, and the body consists of more water and less ash. Since at lowered salinities, *Asterias* reaches osmotic equilibrium with the bathing fluid, it is possible that the reduction in internal salt concentration affects the activity of the enzymes in the cells (Schlieper, 1956). There may also be a dilution of the enzymes since the ratio of water to ash increases at lower salinities.

In a study of the fauna of an estuary along the coast of Maine, Topping and Fuller (1942) report that *Strongylocentrotus dröbachiensis* was found only where the salinity was just slightly less than in the sea. There appear to be no records of echinoids as resistant to low salinities as the asteroid *A. rubens*.

S. purpuratus on the California coast is probably exposed to air[3] in shallow pools for two- to three-hour periods a few times a year, mainly at the low tides of winter and summer. The exposure period is too short to allow sufficient evaporation to make the tide-pool water hypertonic. However, exposure to rain at low tides during the winter and spring months (December to April) may result in significant dilution of the sea water.

Dilution is not general, since the local shore water tested over the year varies only slightly (28.7‰ to 34.18‰ at the Hopkins Marine Station, according to Feder, 1956). Considerable dilutions do occur, however, when torrents of land wash pour into isolated sea urchin tide-pools at low tides during and after heavy rainfall. On December 23, 1955, records indicate 2.7 inches of rain within 24 hours at Carmel, California. During the same period there were low tides of 0.0 and minus 0.7 foot magnitude. The sea urchins were exposed to nearly 50% sea water for as long as two to three hours. On February 24, 1958, titrations from similar pools after a rainfall of 0.72 inch (low tide of plus 0.7 foot) indicated a dilution to 72% sea water. It is therefore not surprising that the purple sea urchin is able to survive exposure to variations in the tonicity of sea water and is capable of withstanding, for a few hours, considerable dilution of the surrounding sea water. In nature, exposure to diluted sea water is probably always brief, since mixture with the main sea water mass quickly restores a concentration close to normal.

Because the purple sea urchin spawns on the California coast during the rainy season, legend has associated spawning with dilution of the sea water, although no proof of a causative relationship between the two is available. Spawning, however, does occur during this season and the eggs are fertilized and may develop for periods of time in diluted sea water. Since a small degree of dilution of the sea water does not affect development of the eggs, the rains probably do not critically affect survival of the embryos developed in nature during this time.

It might be argued that the resistance of developmental stages of *S. purpuratus*

[3] A. Klein and R. Rasmussen (unpublished) found an appreciable loss in weight and an increase in osmotic pressure of the body fluid of sea urchins exposed to air for 24 to 48 hours.

to dilution (or concentration) of sea water represents an adaptation enabling the sea urchin to survive the changes in tonicity it meets in its environment. A similar resistance of the eggs of the echiuroid worm, *Urechis caupo*, to variations of concentration of sea water, comparable to what may occur in its native habitat, has also been recorded (Giese, 1954) and might also be considered an adaptation permitting survival. However, some unpublished studies with the eggs of the deep sea urchin, *Allocentrotus fragilis*, indicate that development in this species is about as resistant to dilution or concentration of the sea water as is *S. purpuratus*. Thus, the delay in cleavage of *A. fragilis* eggs caused by variation in concentration of sea water is about the same as that described above for *S. purpuratus*, and delayed blastulae developed at 150%, 120%, 80% and 70% sea water, becoming progressively fewer in number and more abnormal the greater the deviation from sea water. Normal blastulae, comparable to controls in 100% sea water, were obtained in 90% and 110% sea water. Yet *A. fragilis* lives in deep water (Boolootian *et al.*, 1959) where appreciable changes in salinity are not recorded (Sverdrup *et al.*, 1942). It must be remembered, however, that the larvae of this organism are pelagic (Moore, 1959) and may possibly be exposed to significant variations in salinities since they breed in winter (Giese, 1961) when rains are heavy. However, tolerance, by gametes and other cells of the purple sea urchin, of changes in the osmolality of the medium is perhaps only a measure of the general tolerance of such changes by cells of most marine organisms.

Summary

1. The west coast purple sea urchin, *Strongylocentrotus purpuratus*, was found to be resistant to dilutions and concentrations of sea water for a brief time (three hours) within the range 70% to 120% sea water.

2. The sea urchins resist exposure for many days (35) in 80% to 110% sea water, inclusive. Damage at higher and lower concentrations of sea water is indicated by loss of activity, loss of pigment and appendages, and failure to respond to food, probing, and light.

3. The sea urchins lose weight in hypertonic solutions and gain weight in hypotonic solutions, presumably losing and gaining water, respectively. They recover nearly normal weight after replacement in sea water.

4. The respiration of the sea urchins is little altered by changes in tonicity of the sea water, except at extremes of tonicity where the metabolic rate is decreased.

5. Sea urchin eggs develop normally over the range 90% to 110% sea water, and considerable development occurs over the range 70% to 150% sea water.

6. Gastrulae placed into various concentrations of sea water tolerate the change no better than developing eggs; differentiation proceeds in 90% to 110% sea water, but is essentially stopped at higher or lower concentrations even though the gastrulae survive.

7. Addition of sucrose to 50% sea water to make it to up to 0.5 molal resulted in a medium as favorable as sea water to early cleavage and early development of the sea urchin egg. Addition of sucrose to sea water to make it up to 0.5 molal was as deleterious as 150% sea water to cleavage and early development of the sea urchin egg.

8. Relating the results obtained with sea urchin embryos and adults, it is apparent that the sea urchin is capable of withstanding the maximal variations in the salinity of its natural environment.

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